

CLEAN COPY OF AMENDMENTS TO THE SPECIFICATION

#14/B

In the specification:

The word "protein" is deleted at designated locations throughout the specification so that the sentences wherein such word is found read without the specified word "protein".

Additionally, the amendments include minor typographical errors and replacement with correct words in certain instances as follows:

1. Page 12, sentence beginning line 24 to 27 is amended:

B1  
Moreover, the current invention further provides for proper orientation of each of these molecules within the artificial APC membrane by a novel use of an anchoring mechanism comprising GM-1 and the  $\beta$  subunit of cholera toxin.

2. Page 12, sentence beginning line 29 to page 13, line 1 is amended:

B2  
By attaching the cholera toxin subunit to the molecule of interest, the cholera toxin may be bound by the GM-1 that is incorporated into and has affinity for the nonpolar region of the artificial APC membrane.

3. Page 17, sentence beginning line 11 to 14 is amended:

B3  
There is no relation inherent or otherwise to the current invention, nor is there insight disclosed as to liposome construction containing co-stimulatory and adhesion molecules or protein orientation mechanisms such as the binding of cholera toxin by GM-1, or fused or linked moieties to the MHC, functional or accessory proteins of interest.

4. Page 22, sentence beginning line 9 to 11:

B4  
In a preferred embodiment, the cholera toxin moiety remains in the APC's interior by binding to GM-1 that is incorporated into the APC's lipid interior.

5. Page 22, sentence beginning line 29 to page 23, line 3, amended :

B5  
In still another embodiment, the APC comprises labels wherein a label is associated with at least one of the group selected from the group consisting of a lipid bilayer of the liposome components, a lipid of the liposome, an antigen, an MHC molecule, a co-stimulatory molecule,

an adhesion molecule, a cell modulation molecule, GM-1, cholera toxin  $\beta$  subunit, an irrelevant molecule, and an accessory molecule.

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6. Page 40, paragraph beginning line 17 to page 41, line 8 is amended:

B6 Fig. 27A-C is a schematic showing methodology for orienting molecules of interest in the APC liposome matrix. In 27A, a molecule of interest such as MHC, functional, accessory, adhesion, or irrelevant molecule, may be synthesized by recombinant methods well known to those skilled in the art and linked to GM-1 by a linker and properly oriented in the APC membrane. In 27B, a molecule of interest may be constructed as a fusion protein with cholera toxin  $\beta$  subunit and the fusion protein anchored in proper orientation in the APC membrane by the cholera toxin moiety binding to GM-1. In 27C, a cholera toxin subunit may be chemically linked to SPDP linker (Pierce) and then attached to a molecule of interest followed by anchoring to a GM-1 containing APC. In any of the above, the cholera toxin, GM-1, linkers may be synthetically produced. In the figure, A represents a gene for a molecule of interest, B represents the gene for the  $\beta$  subunit of cholera toxin, A1 is an expression vector, A2 represents expression and isolation of the cloned gene, A3 is an expressed molecule of interest, B1 is a fusion protein of a molecule of interest and cholera toxin, A4 is a linker, A5 is an artificial APC containing GM-1, A7 is a partial view of an artificial APC wherein the molecule of interest is directly linked to the GM-1, C is cholera toxin subunit, C1 is a linker, C2 is a molecule of interest, C3 is a cholera toxin subunit attached to a linker, C4 is a molecule of interest linked to a cholera toxin subunit, E represents a liposome bilayer, E1 shows GM-1, E2 is an artificial APC containing GM-1, and F is a partial view of an artificial APC having a molecule of interest bound to the APC by the binding interaction of the GM-1 and cholera moiety.

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7. Page 42, two sentences beginning line 6 to line 9 are amended:

B7 Other lipid membrane components include GM-1 which is a transmembrane pentasaccharide and associates in part with nonpolar regions of the liposome matrix. This GM-1 can be used in association with cholera toxin  $\beta$  subunit to orient molecules of interest in the liposome matrix.

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8. Page 43, sentence beginning line 3 to line 8 is amended:

8  
With respect to the incorporation of each of the aforementioned MHC, accessory, co-stimulatory, adhesion, modulation, and irrelevant molecules in the artificial APC, proper orientation of these molecule's active centers may be provided by combining the molecules with the  $\beta$  subunit of cholera toxin so that the cholera toxin subunit can be recognized and bound by GM-1 which is incorporated into the liposome membrane matrix.

9. Page 44, sentence beginning line 5 to line 10 is amended:

9  
In still another embodiment, the APC comprises labels wherein a label is associated with at least one of the group selected from the group consisting of a lipid bilayer of the liposome components, a lipid of the liposome, an antigen, an MHC molecule, a co-stimulatory molecule, an adhesion molecule, a cell modulation molecule, GM-1, cholera toxin  $\beta$  subunit, an irrelevant molecule, and an accessory molecule.

10. Page 44, sentence beginning line 23 to line 28 is amended:

10  
In another embodiment, any of the molecules of interest (MHC, accessory, co-stimulatory, adhesion, modulation, irrelevant molecules) can be bound to the  $\beta$  subunit of cholera toxin and GM-1 can be included in the APC lipid matrix to provide a means for proper orientation of the molecules of interest such that their active centers are oriented to facilitate interaction with T cells and other components external to the APC.

11. Page 47, sentence beginning line 17 to 20 is amended:

11  
Moreover, the lipid bilayer may also include accessory molecules such as cholesterol to provide elasticity in the bilayer and GM-1 to provide an anchor for orientation of cholera  $\beta$  subunit comprising molecules of interest.

12. Page 49, sentence beginning line 28 to 30 is amended:

12  
Further, GM-1 is incorporated into the lipid layer matrix providing a means by which the cholera toxin portion can be bound and the molecule of interest properly oriented in the lipid layer.

13. Page 93, the sentence beginning line 9 and ending line 12 is amended:

B13  
To visualize free movement of the TCR in the T cell membrane, we employed a system where FITC-conjugated cholera toxin, a molecule known to combine with the intracellular portion of transmembrane molecules, is introduced into the T cells.

14. Page 94, the sentence beginning line 4 is amended:

B14  
In Fig. 16A-D, we visualized comigration of the cholera raft and the TCR, by incubating T cells with PE-conjugated monoclonal antibody (Pharmigen).

15. Page 94, the sentence beginning line 12-14 is amended:

B15  
In experiments described in Fig. 18A-D, we visualized the TCR using an Alexa red-conjugated anti CD3 monoclonal (Molecular Probes, Eugene OR) and the liposomes using FITC-conjugated streptavidin which bound to the biotin at the N-terminal of the OVA peptide.

16. Page 104, two sentences beginning line 7 to line 11 are amended:

B16  
This mechanism uses GM-1 pentasaccharide which is a transmembrane molecule and has an affinity for binding the  $\beta$  subunit for cholera toxin. When the GM-1 is associated with the liposome membrane of the APC, it can be used to bind cholera toxin which in turn can be attached to the molecule of interest.

17. Page 105, sentence beginning line 7-8 is amended:

B17  
Since the  $\beta$  subunit is primarily involved in the binding to the GM1 pentasaccharide, the  $\alpha$  subunits are not necessary.

18. Page 106, sentence beginning line 6 to line 7 is amended:

B18  
The GM1 is a transmembrane molecule which can be associated with the "raft" or freely mobile molecules of interest in the lipid membrane.

19. Page 106, sentence beginning line 12 to line 13 is amended:

B19  
The recombinant product can be purified and linked to GM-1 that is in an artificial APC.

20. Page 106, two sentences beginning line 15 to line 21 are amended:

BD  
The fusion product can be purified and mixed with an artificial APC containing GM-1 where the cholera moiety will bind to the GM-1. Additionally, as shown in Fig. 27C, the cholera toxin (whether natural or recombinant) can be attached to a linker, such as N-succinimidyl [3-(2-pyridyl) dithio] propionate, either to a complete  $\beta$  subunit molecule or during synthesis of a recombinant toxin molecule, the product of which can be then mixed with a GM-1 containing artificial APC.

- BD  
21. Page 106, sentence beginning line 24 to line 26 is amended:

Once the GM1 is incorporated into the liposome of the APC, cholera toxin-cojugated surface proteins can then be cross-linked.